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Embryo-toxic Effects Produced by Magnesium Deficiency in Rats

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During pregnancy the Mg-concentration in the serum of rats on a Mg-rich diet showed no significant decrease, but the intracellular Mg content increased in the liver, muscle and heart. In pregnant rats on Mg-deficient diets, resorption and retardation of fetuses were observed, and to a lesser extent, malformations of the extremities and the abdominal-wall of fetuses also occurred. The systematic variation of the onset and duration of Mg-deficient-feeding demonstrated that the most marked effect is seen when the serum Mg-concentration of the mother animal is decreased on day 10—11 of pregnancy.
In Mg-deficient fetuses the Mg-content was slightly decreased, the K-content was greatly decreased, while the Na-content was increased.
In the electron microscope, the cells of the fetuses which were undergoing resorption showed membrane fragmentation and pycnosis of the nuclei.
New born rats of mother animals which had received a Mg deficient diet from day 5—12 of pregnancy showed a high death-rate. By histological examination we found a swelling of the mitochondria and a loss of lipid inclusions in the brown adipose tissue. In the brain there were necroses which showed no definite glia-reactions, a decrease in the number and thickness of the layers in the cortex and a hydrocephalus externus. Other organs were morphologically unchanged.

Während der Schwangerschaft sank die Mg-Konzentration im Serum Mg-reich ernährter Ratten nicht signifikant ab. Der Mg-Gehalt in Leber, Muskel und Herz nahm zu. Wurden schwangere Ratten Mg-arm ernährt, wurden Resorptionen, Retardation und in geringem Ausmaß Mißbildungen der Extremitäten und der Bauchwand beobachtet. Bei systematischer Variation von Beginn und Dauer der Mg-armen Ernährung ergaben sich die stärksten Effekte, wenn die Mg-Konzentration im Serum der Muttertiere am 10.—11. Schwangerschaftstage erniedrigt war.
In den Mg-Mangel-Feten war der Mg-Gehalt geringfügig und der K-Gehalt stark vermindert, während der Na-Gehalt erhöht war. Die Zellen der Foeten im Zustand der Resorption zeigten Fragmentierung der Membranen und Kernpyknose.
Neugeborene Ratten, deren Mütter von Tag 5—12 der Gravidität Mg-arm ernährt wurden, wiesen eine hohe Sterblichkeit auf. Wir fanden histologisch Mitochondrienschwellung und Verlust der Fetttropfen im braunen Fettgewebe. Im Gehirn traten Nekrosen ohne Gliareaktion auf. Es zeigte sich eine Abnahme der Zahl und Dicke der Hirnschichten sowie Hydrocephalus externus. Die anderen Organe waren morphologisch nicht verändert.

Considerable evidence has been accumulated that a deficiency of amino acids, vitamins or trace elements is able to produce embryotoxic effects in experimental animals (1, 2, 3). But the consequences of a magnesium deficiency in the maternal organism on embryonic development have not yet been elucidated in detail. Since it is known that the concentration of magnesium is decreased during pregnancy in women (4, 5) it appears possible that a latent magnesium deficiency which may occur in men under conditions of an unbalanced food-intake could be exaggerated during pregnancy so that the embryo is confronted with a deficient supply of magnesium (for review 5, 6).
We have studied the effect of a maternal magnesium deficiency on embryonic development of rats in some detail. Since it is well known that magnesium represents an essential co-factor in many enzyme reactions, including nucleic acid and protein synthesis reactions, embryonic tissue may provide a suitable model for studying the effect of magnesium deficiency on enzyme patterns, as well as on growth and differentiation processes. The rat was chosen for these studies because the change in magnesium concentration in blood and different tissues during magnesium deficiency is well known (7) and at the same time we have some information on the morphological and biochemical changes occurring during embryonic development in this species.

Experimental Procedure

Wistar rats of the strain SW 69 weighing 180 g were used for the studies. The animals were mated for a 2 hrs period and the following 24 hrs were called day 0 if sperm could be detected in the vaginal smear. The animals were fed an artificial Mg-rich and Mg-deficient food for different periods. The composition of the food has been described (7). The experimental series investigated are summarized in Table 3. The pregnant rats of each group were sacrified on day 20 and the embryos removed, weighed and checked for macroscopic malformations. These fetuses were fixed in 100 g/l formaldehyde, stained with alicarine and cleared with Na OH.
The methods for measuring Mg, Na and K-contents in the tissues have been described before (7). For the estimation of Mg in serum, 0.1 ml of serum was deproteinized with 2 ml of a solution containing 100 g/l trichloroacetic acid and 1 g/l Lanthanum as $LaCl_3$.
For light and electron microscopical studies the embryos or new born rats were fixed in Karnovsky's solution (3% glutaraldehyde and 3% paraformaldehyde in 0.1 mol/l cacodylate buffer, pH 7.2). For light microscopical examinations the embryos had to be additionally fixed in Karnovsky-solution before embedding in paraplast. They were stained with hematoxylin eosin as well as

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by the PAS- and Trichrome-methods. For the electron microscopical studies the fixation in KARNOVSKY was followed by dehydration with acetone and an embedding in micropal (Firma Ferak Berlin). Butting was performed with a LKB-Ultrotome and the sections were contrasted with uranylacetate-lead acetate. A Siemens Elmiscope I and Ia were used.

Results

General behaviour

Symptoms of magnesium deficiency usually appear after more than one week on a Mg-deficient diet, so that up to one week of Mg-deficiency, experimental animals showed no typical symptoms. The groups fed the Mg deficient diet for two or three weeks showed the Mg deficient symptoms to a lesser degree than non pregnant animals; i. e., redness of the ears and scrawls, but no necroses at the ears and no cramp.

Magnesium metabolism

The magnesium concentration in the serum of pregnant rats under our conditions of Mg nutrition showed no significant decrease in contrast to some observations in women. This may be due to the high Mg-content of our normal diet.

During the 8 days following the commencement of the magnesium depleted diet, the magnesium concentration of the serum declines from 1.07 mmol/l to 0.3 mmol/l and then stays about constant at about 0.23 mmol/l during the experimental period (Fig. 1). This decrease is more rapid and reaches a lower value than in non pregnant animals during Mg-deficiency, which shows the higher Mg-requirement in pregnancy.

The magnesium content of some of the maternal tissues, especially liver, heart and muscle, increased during pregnancy. This intracellular magnesium accumulation is also observed in liver and muscle when the magnesium concentration in serum is drastically reduced by a magnesium depleted diet (Tab. 1).

In 12 day old rat embryos, the mothers of which had been fed a magnesium depleted diet from day 0—12, changes in electrolyte metabolism characteristic for magnesium deficiency could be observed: in addition to a decline in the magnesium content we found a pronounced decrease in the potassium concentration and a small increase in the sodium content (Tab. 2).

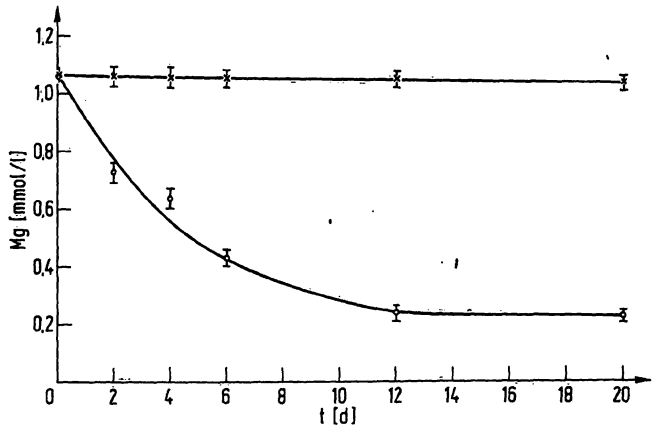

Fig. 1
Mg-concentration in serum of rats during pregnancy
× rats fed a Mg-rich artificial food (7)
O rats fed a Mg-poor artificial food (7)

Tab. 2
Electrolyte contents from 12 day old embryos whose mother-animals were fed Mg-poor food from day 0—12. The mean ± standard error of the mean was determined from 6 fetuses of 3 mother animals in each group. DNA was estimated according to SCHMIDT, G. and THANNHAUSER, S. J. (1945), J. Biol. Chem. *161*, 83—89

	Na	K	Mg
		[µmol/mg DNA]	
Control-fetuses	31.8 ±1.0	24.4 ±0.2	1.42 ±0.04
Mg-deficient-fetuses	33.4 ±0.6	15.4 ±0.5	1.11 ±0.05

Macroscopical Results

Rate of resorption

In some of the experimental series the rate of resorption was very high. The results are summarized in Table 3. It may be seen that fetal lethality of approximately 100% is obtained if the magnesium deficient diet is

Tab. 1
Mg contents in different organs. Pregnant rats were fed Mg-rich [for ingredients see (7)] or Mg-poor (Mg-poor laboratory-food) diets for various periods. The mean ± standard error of the mean was determined from 6 animals respectively

	Day 0	Day 3	Day 6	Day 12	Day 18	Day 20
	Mg content (mmol/kg dried substance)					
Liver Mg-rich food	28.51 ±0.25	27.76 ±0.50	30.14 ±0.02	29.92 ±0.07	32.32 ±0.87	33.38 ±0.15
Liver Mg-poor food	27.95 ±0.27	27.06 ±0.54	29.80 ±0.29	27.90 ±0.20	29.40 ±0.47	31.30 ±0.74
Muscle Mg-rich food	43.77 ±0.20	45.05 ±0.15	44.63 ±0.27	45.30 ±0.20	45.52 ±0.47	45.65 ±0.55
Muscle Mg-poor food	43.48 ±0.19	42.23 ±0.43	44.80 ±0.32	45.11 ±0.32	44.96 ±0.26	45.83 ±0.22
Kidney Mg-rich food	32.39 ±0.30	31.57 ±0.08	32.11 ±0.60	32.64 ±0.28	32.63 ±0.92	32.69 ±0.03
Heart Mg-rich food	37.57 ±0.15	37.95 ±0.15	37.93 ±0.46	37.70 ±0.40	38.97 ±0.72	39.70 ±0.30
Bone Mg-rich food	161.93 ±1.29	165.25 ±1.74	164.66 ±1.54	158.75 ±1.74	157.75 ±2.00	164.62 ±1.24

Tab. 3
Resorptions and malformations of fetuses from rats that had been placed on Mg-deficient food

Group	Mg-deficient nutrition (first—last day)	Number of litters	Number of implantation sites	Resorbed fetuses in different litters*	Resorptions %	Malformed fetuses
Control	—	10	94	3	3	—
1	1—20	2	13	8.5.	100	—
2	1—13	5	37	8.8.8.7.6.	100	—
3	5—15	5	39	10.8.8.8.4.	97	—
4	6.5—11	5	43	—	0	—
5	7—15	4	35	9.9.8.1.	77	2
6	8—14	8	83	10.7.1.1.	23	—
7	8—12	7	70	5.3.1.	13	—
8	10—17	7	65	2.	3	1
9	10—15	7	70	1.	1	—
10	12—20	4	46	2.1.	3	—
11	12—17	7	81	1.1.	3	—

* Each figure gives the resorptions in one rat.

given during the whole pregnancy (day 0—20) or from day 1—13 p. c. and day 5—15 p. c. respectively. An analysis of the systematic variations of the magnesium deficient food period reveals that the rate of resorption is always high when the magnesium concentration in the serum of the mother animals is low at day 11 of pregnancy. To reach such low values, the magnesium deficient food must be given for 5—6 days (Fig. 1). If this period is too short, the magnesium concentration in the maternal serum does not decrease enough to allow an embryo-toxic effect. If the magnesium deficient diet is started later than on day 10 of pregnancy, the number of resorptions does not differ significantly from that of controls. When the magnesium deficiency was reached at about day 13 of pregnancy, the resorptions in general were confined to a few mother animals while others show very few resorptions.

Wet weight of fetuses

The weights of fetuses obtained by Caesarian section on day 20 of pregnancy are summarized in Table 4. While in the experimental group 1—3 no viable fetuses were observed, there was a significant decline in the average wet weight of the fetuses from the experimental groups 4, 5, 7 and 8. No significant deviation from the controls was observed when the magnesium deficient food was given during days 8—14, 10—15, or 12—17.

Malformations

Surprisingly, very few malformations were observed in our studies. The rate of spontaneous malformations in our strain is only 0.3%. There were two malformations in the experimental group 5 and one in the experimental group 8. We feel that further experiments are necessary to elucidate this question, because these results don't agree with those of HURLEY (5).

Two malformations affected the extremities, in one case the two upper, in the other all extremities (dysmelies) were shortened. During the clearing and Alizarin-red-staining, a marked shortening of the long bones of the extremities was found, but all the important skeleton-

Tab. 4
Wet weights of fetuses and placentae from rats that had been placed on Mg-deficient food for different time intervals during pregnancy

Group	Mg-deficient nutrition (first—last day)	Fetuses examined	Wet weights [g] of fetuses $\bar{x} \pm s_{\bar{x}}$	Significance of wet weights to controls
Control	—	91	3.23 ±0.26	—
1	1—20	—	—	—
2	1—13	—	—	—
3	5—15	—	—	—
4	6.5—11	43	2.75 ±0.42	$p < 0.001$
5	7—15	8*	2.85 ±0.22	$p < 0.001$
6	8—14	64	3.18 ±0.21	$p > 0.05$
7	8—12	61	3.09 ±0.23	$p < 0.001$
8	10—17	63	3.04 ±0.21	$p < 0.001$
9	10—15	69	3.16 ±0.27	$p > 0.05$
10	12—20	43	3.07 ±0.33	$p = 0.01$
11	12—17	79	3.18 ±0.42	$p > 0.05$

* 1 litter.

parts were present (Fig. 2b). The length of the humerus and femur of control embryos are 2.59 ± 0.15 mm and 1.78 ± 0.16 mm respectively. In the malformations the corresponding values were 1.98 mm for the humerus and 1.21 mm for the femur. The third malformation shows a large abdominal fissure of prolapsing viscera (Fig. 2c).

Morphological studies of embryos

In 18 day embryos, whose mothers were fed a Mg-deficient diet from day 1—13 and which were in process of resorption, all celltypes and tissues were clearly damaged. The nuclei are here pycnotic or show a clear karyorhexis. Cell membranes and the membranes of the cytoplasmic organelles are disrupted locally. The adhesion disappears and the epithelium tissues are greatly loosened. With the electron microscope these changes in damaged embryos can be seen and registered more precisely. In the nucleus the clumping and fragmentation of the chromatin is striking. The cell organelles are balooned, fragmented in vesicles or almost lost. Only few cells show an intact cell membrane and in many cases only fragments can be seen (Fig. 3).

Post natal findings

New born rats from mothers fed a Mg-deficient diet from day 5—12 showed a high death rate (Tab. 5). In

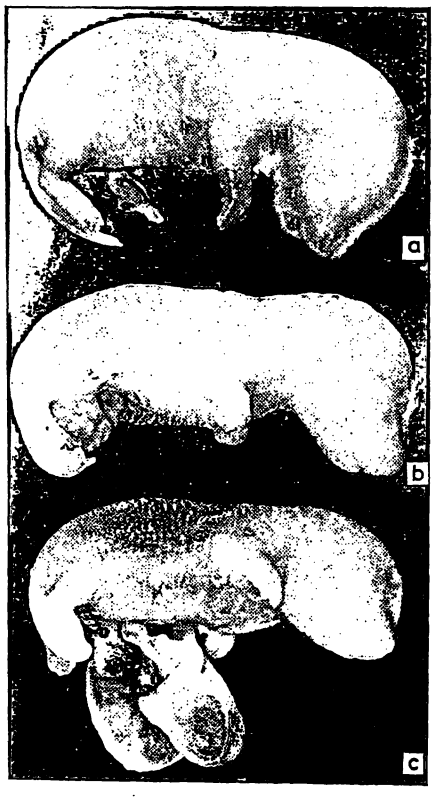

Fig. 2
Rat fetuses of day 20 from group 8, 1/2 natural size
a) Normal fetus
b) Fetus with dysmelies of the upper extremity siren formation behind and an abdominal hernia
c) Fetus with shortened snout and a pronounced defect of the front abdominal wall

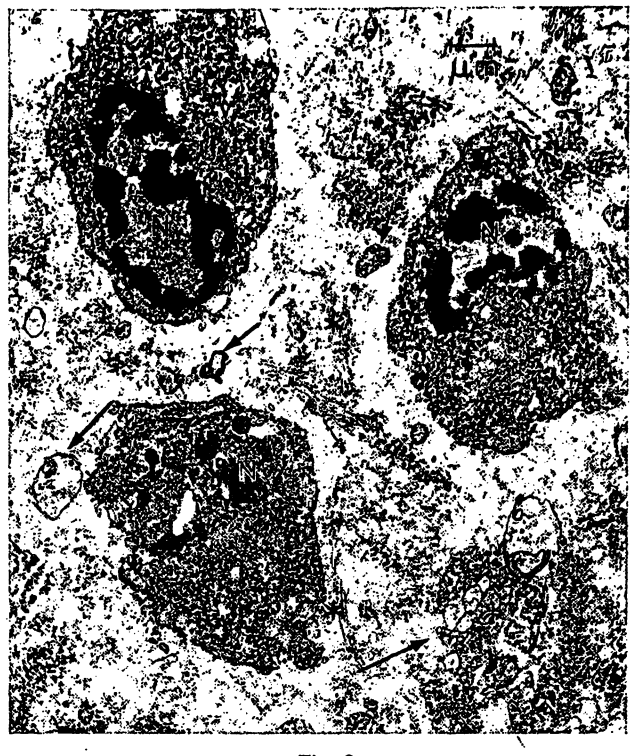

Fig. 3
Mesenchyma-region from the cutis area at the back of a 12-day-old embryo (fed on Mg-deficient diet from day 0). Fragmentated cell membrane and organell membrane. Cell fragments in the extracellular compartment (↙). Some additional small bundles of collagen fibrills (*)
Obvious pycnotic nucleus (N)
Magnification: 1:4600

Tab. 5
Number of new born rats which were dead at birth or died thereafter

Mother animals	Number of new borns living	dead	New borns dying in period day 0—4 post partum	day 4—21 post partum
Control [n = 10]	81	0	2	0
Mg deficient diet day 5—12 [n = 10]	45	8	15	0

similar experiments of WANG et al. (8) up to 90% of the new born rats died within the first week. In this case no pathological findings could be obtained macroscopically. Morphological investigations revealed, however, alterations in the brown adipose tissue and in the brain of the dead animals. The same was found in some animals which had been removed on day 20 by Caesarian section. The lipid inclusions in the brown adipose tissue (Fig. 4) which are very numerous before birth and after day 2 post partum had disappeared. At the same time a clear cut swelling of the mitochondria could be observed. Similar findings can be obtained from adult animals with a chronic Mg-deficiency (9).

Necroses occur in the brain and they are located in the grey matter. Their location, however, is variable so that it is not possible to attribute them to specific brain regions. A pronounced glia cell reaction in the vicinity of the necroses is lacking (Fig. 5b, c). The number of layers and cells, and the thickness of the layers in the

Fig. 4
a) Brown adipose tissue from a rat 3 days after birth with an obvious swelling of the mitochondria (M). No lipid inclusions
N = Nucleus
Magnification 1:28000
b) Brown adipose tissue from a control-rat
Magnification: 1:28000

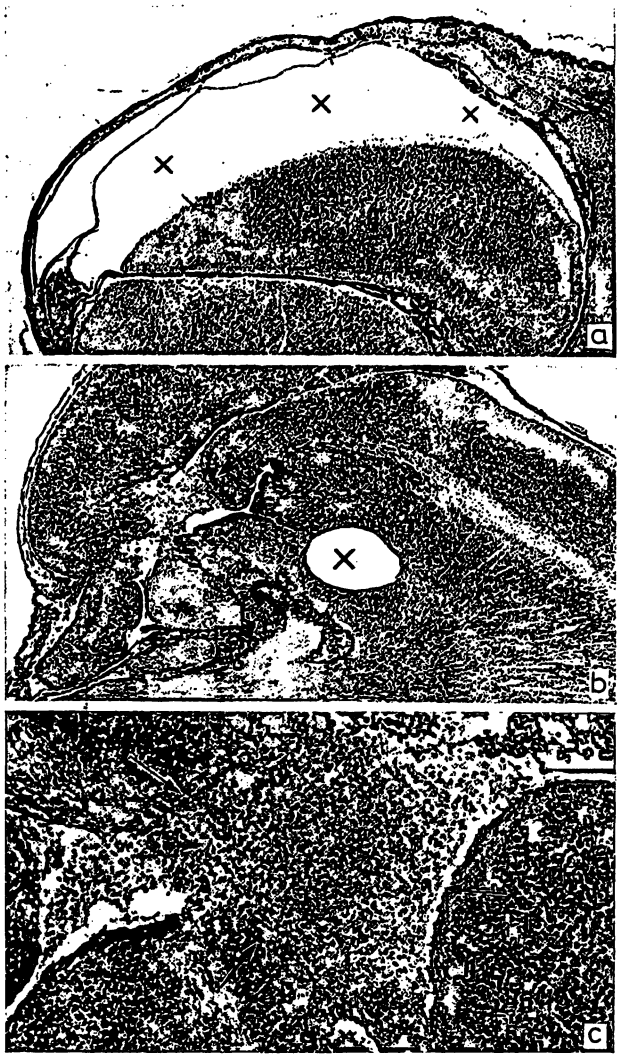

Fig. 5
New born rats (mothers fed on Mg-deficient diet from day 5 to 12)
a) Hydrocephalus externus (×), tangential section through the telencephalon (T)
b) Necrotic area (↙), disturbing the normal construction of the brain in the neighbourhood, × = old necrotic zone, transformed to a vacuole
c) Higher magnification of a similar necrotic zone

region of the telencephalon are reduced. Concomitant with a striking decrease in the thickness of the brain cortex wall, hydrocephalus externus can often be observed (Fig. 5a).

Discussion

During pregnancy there is an increased uptake of Mg in the intracellular compartment which may be produced by an increased level of oestrogen (10, 11). This Mg-uptake also occurs in Mg-deficiency, when the serum Mg-concentration is strongly decreased. Therefore, it follows that during pregnancy Mg is transported into the cells. In Mg-deficient, pregnant rats, this Mg-transport occurs against a higher electrochemical potential than in pregnant and non-pregnant control-animals. As a result of the changed Mg-distribution and the higher Mg-requirement by the developing fetuses the Mg-concentration in the serum can decrease if the Mg-input is not sufficient. Thus, even in humans, a latent Mg-poor nutrition, additional increased Mg-need through protein-rich nutrition, a diminished Mg-resorption in the intestine, e. g., through Ca-rich-nutrition, or increased loss of Mg, through diuresis or alcohol could possibly lead to Mg concentrations that are embryotoxic.

The alterations described in the mineral metabolism of the Mg-deficient fetuses are also found in other Mg-deficient cells and tissues and are probably not a direct consequence of a decrease of the intracellular Mg-ion-activity. As the total Mg-concentration of the fetuses is only little reduced (Tab. 2) and because the intracellular Mg is buffered (12) the intracellular Mg-ion-activity should remain constant.

In Mg-deficiency there is a heavy decrease in the extracellular Mg and in the intracellular K-concentration, and a slight increase in the Na-concentration (13). This is not caused by an alteration of active K-transport but by an increase in the K-efflux from the cell. This is accompanied by a decrease in the rate of synthesis of

DNA and protein (13, 14) and also of lipids in the brain (15). This inhibition of lipid synthesis in Mg-deficiency may be the reason for changes in the brain. On day 11 of gestation the nerve cell processes of the rat begin to grow. This brings about a great increase in cell surface and thus a high demand for membrane lipids. If this process is disturbed by Mg-deficiency some centres may not be connected with one another. Subsequently necroses might occur.

Another cause for the high death rate may be the alterations in the brown adipose tissue. This tissue plays an important role in temperature regulation, especially after birth. In normal new borns immediately after birth the lipid droplets are dissolved and oxidative phosphorylation in the brown adipose tissue is uncoupled. A few days later lipids are again restored (16, 17). The loss of lipid droplets and the swelling of the mitochondria in the brown adipose tissue of Mg-deficient fetuses at day 21 of gestation may indicate that the cold acclimatisation in these animals is disturbed.

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